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Intraerythrocytic Killing of Malaria Parasites

Annual/Final Report

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The purose of these studies was to determine the role of activated macrophages in immunity to the blood stages of malaria. This has been accomplished by comparing the activity of macrophages during lethal and non-lethal malaria infections and in malaria-resistant and non-resistant mice. The results indicate that in non- lethal P.yoelii infection of mice, macrophage activity, as determined by H_2O_2release, gamma-interferon production and lymphoproliferation, is high early in infection, declines during the time of peak parasitemia and recovers as the infection is cleared. Although some macrophage functions are enhanced during the early phases of lethal P. yoelii infection, this enhancement is not sustained. Recombinant gamma-interferon, injected into mice, had a protective effect in susceptible mice with lethal infections but not in non-lethal infections.

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Summary

The purpose of these studies was to determine the role of activated macrophages in immunity to the blood stages of malaria. This was accomplished by comparing the activity of macrophages during lethal and non-lethal malaria infections and in malaria-resistant and non-resistant mice.

We have found that spleen cells from mice infected with non-lethal Plasmodium yoelii have higher ADCC levels than those with a lethal infection. Our studies further showed that in Balb/cByJ mice, which are relatively susceptible to PyL and PyNL, there was an initial burst of H_2O_2 release and gamma-IFN production only in response to PyNL whereas in CBA/J mice which are relatively resistant to these parasites, there was an initial burst of H_2O_2 and gamma-IFN in response to both PyL and PyNL. The kinetics and the lymphoproliferative response to P. yoelii antigens corresponded to that of H_2O_2 and gamma-IFN production. In all infections, levels of gamma-IFN declined as parasitemia increased; however, non-lethal infections were characterized by a recovery of both gamma-IFN activity and H_2O_2 release as parasitemia declined. Further, administration of recombinant murine gamma-IFN (Genentech, Inc.), resulted in a dose-dependent protection of SW, Balb/cByJ and CBA/J mice from the lethal variant of Plasmodium yoelii 17x (PyL) but had little effect on the course of the non-lethal variant of this parasite (PyNL). Administration of recombinant gamma-IFN also resulted in the activation of peritoneal macrophages for increased phagocytosis of malaria-infected erythrocytes and release of H_2O_2, as measured in vitro.

In related studies, we described a 96kd antigen, present in the erythrocytes of P. chabaudi-infected erythrocytes which appears to be partially protective and which also cross-reacts with P. falciparum, P. vivax and P. cynomolgi. We also studied malaria in B thalassemic mice and found them to be partially resistant to P. chabaudi.

We conclude that cellular immunity, especially macrophage function is an important element in the immune response to malaria.

Foreword

Citations of commercial organizations and trade names in the report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978)".

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Fig. 1. Lymphoproliferative responses to PyL antigen of Balb/c ByJ and CBA/J mice during PyL and PyNL.

Scientific Report

Research Problem

Evidence from our laboratory indicated that lymphokines (LK), which are produced by the spleen during rodent malaria infections, stimulate normal, resident macrophages to bind and ingest and to kill intraerythrocytic malaria parasites in vitro (1, 2).

The killing of the intraerythrocytic parasites occurs across a 0.45 um membrane and appears to be mediated by H_2O_2 secreted by the macrophages (2). Killing is enhanced by a phagocytic stiumulus to the activated macrophages (2). Human gamma-IFN (Genentech, Inc.) can activate human monocyte-derived macrophages to kill P. falciparum (3) and preliminary evidence suggested that LK obtained from malaria-infected mice also contain gamma-IFN.

We hypothesized that the intraerythrocytic killing of malaria parasites by macrophages is an important effector mechanism in this disease. The purpose of these studies was to determine whether this is so by determining the role of macrophage-mediated intraerythrocytic parasite killing and other related macrophage activities in several in vivo models of malaria.

Background

Much evidence is accumulating to indicate that cell-mediated immune responses are very important in the response to some species of malaria (4) and may play an important part in the resistance of vaccinated mice. However, the actual mechanisms whereby cell-mediated responses protect against malaria are not known.

The overwhelming evidence supports the concept that activated macrophages are involved in controlling several infections such as Leishmania tropica (5), Rickettsia akari (6), and Trypanosome cruzi (7); that they are cytotoxic to tumor cells (8), and finally that they may be involved in vaccine-induced immunity.

We have been studying macrophage activation during rodent malaria (9) and the effects of macrophages activated with other stimulants on malaria-infected erythrocytes (1). We found that spleen cells of mice infected with BCG or malaria, produced factors or lymphokines (LK) which stimulated normal mouse peritoneal macrophages for enhanced phagocytosis of parasitized erythrocytes (1) and for killing of parasitized erythrocytes (2). We also found that fresh monocytes or LK-stimulated, monocyte-derived macrophages were active in inhibiting the multiplication of P. falciparum (3). In both systems H_2O_2 seemed to be the active molecule. Killing was observed after parasitized erythrocytes bound to monocytes and was associated with an oxidative burst in the monocytes. After the

interaction, the parasitized erythrocytes appeared to be degenerating and looked like the previously described "crisis" forms (10).

Our results confirmed and extended several other findings. Taliaferro and Cannon (24) observed that upon acquisition of immunity in monkeys infected with malaria, some intraerythrocytic parasites appear to degenerate within the erythrocytes. This finding implied that soluble mediators might affect malarial parasites. That such mediators might be secreted by macrophages was first suggested by Allison and Clark (11). Mice treated with BCG are protected against malaria and the mechanism suggested was that parasites are killed by products of activated macrophages. Since then, other parasiticidal factors have also been shown to have an effect on intraerythrocytic malaria parasites, namely, tumor necrosis factor (12), interferon (12) and a lipopolysaccharide-induced serum factor (13).

The concept that oxygen radicals might affect malaria parasites was suggested by the observations that injections of alloxan (14) and t-butyl hydroperoxide (15,16) into mice with P. vinckei (14, 16) or P. yoelii (15) markedly reduces parasitemia. These compounds generate reactive oxygen intermediates and their activity can be inhibited by iron-chelating agents such as desferrioxamine and diethylthiocarbamate. It is well known that malaria parasites are sensitive to oxidant stress. Cultures of P. falciparum grown in G-6-PD deficient erythrocytes are inhibited under high oxygen tension (17). In addition, dilutions of H_2O_2 as low as 10^{-5}M are toxic to P. yoelii and P. berghei in vitro and in vivo (18).

Early studies of Langhorne, et al (19) indicated that incubation of spleen cells from infected monkeys with parasitized erythrocytes reduced their ability to multiply. Later, Taverne, et al (20) demonstrated the killing of P. yoelii by cells of the monocyte-macrophage series. Data from this study also suggested that fresh blood monocytes or peritoneal cells activated by incubation with lymph node cells of immunized mice were more effective than normal peritoneal cells. Our studies showed that H_2O_2, produced upon an oxidative burst in activated macrophages, is lethally damaging to P. yoelii and P. falciparum. Because these studies suggested an important protective mechanism in malaria, we felt that further studies in animal models to determine the in vivo relevance of this mechanism were warranted.

Approach

We approached this by studying:

1. The ability of splenic macrophages from mice infected with lethal and non-lethal malaria to mediate the killing of intraerythrocytic malaria parasites via antibody-dependent cell-mediated cytotoxicity (21).

2. a) The ability of spleen cells from mice infected with lethal and non-lethal malaria to secrete H_2O_2 and gamma-IFN and proliferate in response to Plasmodial antigens. These activities were studied in both malaria-susceptible and resistant mice (22).

2. b) The effect of gamma-IFN on lethal and non-lethal malaria (22).

3. The role of Pc96, an antigen of P. chabaudi in the erythrocyte membrane of infected mice in cell-mediated immunity (23, 24) and its relevance to human malaria (25).

4. The effect of the B-thalassemia mutation on malaria-infected mice and the role of the spleen in this infection (26).

Results

1. Antibody-dependent, cell-mediated cytotoxicity in lethal and non-lethal malaria (21).

ADCC mediated by spleen cells from normal mice and mice infected with either the nonlethal (17xNL) or lethal (17xL) variant of P. yoelii is shown in Fig. 2 (21).

Significantly more lysis was obtained on day 6 with spleen cells from mice infected with the nonlethal variant (parasitemia, 1.5%) than with spleen cells obtained from normal mice ($P<0.01$). In addition, there was a decline in cytotoxicity later in the course of P. yoelii 17xNL infection (parasitemia on day 12, 12.5%) similar to that observed during P. berghei infection, (21, Fig. 1). In contrast, cytotoxicity of spleen cells from mice infected with P. yoelii 17xL (parasitemia, 8.5% on day 6 and 56% on day 12) was inhibited compared with that of cells from normal mice ($P<0.01$).

Since ADCC is clearly enhanced at some points during malarial infection and with some parasites, it was important to determine whether parasitized erythrocytes would be lysed in this assay. Furthermore, both human (27) and rodent (28) infected erythrocytes have been shown to be coated with IgG, which might mediate ADCC. In several experiments, P. berghei-infected erythrocytes could not be lysed by activated peritoneal macrophages, even after the infected cells were presensitized with hyperimmune serum. However, when P. berghei-infected erythrocytes were presensitized with rabbit anti-mouse erythrocyte IgG, they did release ^{51}Cr, although less than that released by moEIgG (21, Table 1). This result could reflect lysis of only uninfected erythrocytes; however, since the amount of ^{51}Cr released was reduced by less than 50% compared with that from normal mouse erythrocytes, this explanation is unlikely.

2. a) Ability of spleen cells from mice infected with lethal and non-lethal malaria to 1.) secrete H_2O_2, 2.) secrete gamma-IFN and 3.) proliferate in response to P. yoelii antigens.

Our next objective was to determine whether there was difference in the ability of macrophages from mice infected with lethal and non-lehtal Plasmodium yoelii infection to produce H_2O_2.

1. H_2O_2 release studies.

The first series of experiments was performed on female SW mice 6-10 weeks old. Animals were infected with 10,000 infected erythrocytes. At several time points, mice were sacrificed, the peritoneal cavity rinsed, and peritoneal cells assayed for H_2O_2 release after triggering with PMA (23). Our results showed that peritoneal macrophages from mice infected with the non-lethal strain begin to produce increased levels of H_2O_2 at approximately day 4 of infection. Peak amounts of H_2O_2 are produced by approximately day 9 and then the levels decline as parasitemia declines. In contrast, macrophages obtained from mice infected with the lethal strain of P. yoelii did not show an increase in H_2O_2 production until day 7.

These findings were pursued in mice that were either relatively susceptible or relatively resistant to malaria. H_2O_2 responses of splenic macrophages were measured throughout the course of infection (22, Fig. 5). In Balb/c ByJ mice infected with PyNL, there was an initial increase in H_2O_2 release in response to PMA during the first few days of infection ($P<0.05$). This response also declined as parasitemia rose but began to recover by day 20 of infection. On the other hand, in mice infected with PyL, there was only an initial burst of H_2O_2 by spleen macrophages. Levels of H_2O_2 then declined and there was no recovery.

In the more resistant CBA mice, there was an initial burst of H_2O_2 release, and then a decline to well below control levels. However, on day 20 of infection, H_2O_2 levels began to increase and continued to increase further until the infection was cleared. The pattern of the H_2O_2 response in CBA/J mice infected with the more virulent parasite was almost the same as with the mild parasite. There was an initial burst of activity, a decline and then a recovery. The recovery was not as great as in the mild infection, however (Fig. 5).

2. Gamma-IFN production.

Because gamma-IFN is a well-known macrophage activator (30), we wanted to know whether the increased macrophage activity observed during malaria (cytotoxicity, H_2O_2 release) was due to increased production of gamma-IFN during infection. We found that in

Balb/cByJ (susceptible) mice infected with PyNL, there was an early burst of gamma-IFN in response to soluble malaria antigens (31), as determined using spleen cells obtained on day 4 and 5 of infection ($P<0.05$, Fig. 4) (22). As parasitemia increased, however, gamma-IFN levels declined to control levels. In contrast, in the same strain of mice infected with PyL, there was no initial burst of gamma-IFN and levels of the lymphokine barely exceeded control values at any point during infection.

In CBA/J mice (resistant), there was an initial burst of gamma-IFN production in spleen cells from mice infected with both the non-lethal and the lethal variants of Py17x ($P<0.001$). Sinmilar to the observations in Balb/c mice, levels of gamma-IFN declined as parasitemia increased. However, as the infection resolved, animals infected with both L and NL parasites produced a second burst of the lymphokine ([illegible], Fig. 4, 22).

3. <u>Lymphoproliferation in response to P. yoelii antigen</u>. (Srinivasan, [illegible], H., in preparation).

Lymphoproliferative responses to P. yoelii erythrocytic antigens were measured in rder to relate lymphocyte activation to H_2O_2 and gamma-IFN release. In Balb/c mice (susceptible), there was an early proliferative response to both lethal and non-lethal infections. Responses fell to below control levels as parasitemia peaked. In mice infected with Py17x NL, proliferative responses began to recover on day 20. A similar pattern was observed in CBA/J mice infected with Py17xNL. Curiously, in CBA/J mice infected with Py17xL, there was a continuous increase in the lymphoproliferative response until day 17 and then a decline to control levels (Fig.1, Appendix item #1).

2. b) <u>Effect of recombinant gamma-IFN on the course of lethal and non-lethal malaria</u>.

Another way of determining whether macrophage activation was important in the *in vivo* models of infection was to see the effect of injecting gamma-IFN on the course of the disease.

As shown in Fig. 1A (22), injection of 10,000 U of gamma-interferon/day had a protective effect on SW mice infected with PyL, as shown by their diminished parasitemia from day 5 on, ($P<0.001$) and the increased survival of the mice (average day of death = 9.5, compared with 8.5 for mice injected with diluent or thioglycollate). Animals which received 50,000 U/day and 100,000 U/day had an even more pronounced improvement in the course of parasitemia and greater survival. Animals receiving 50,000 U/day survived a mean of 11.5 days and mice receiving 100,000 U survived a mean of 13.7 days. In addition, one mouse which received 50,000 U/day and on. mouse which received 100,000 U/day did not become patent at all. These two mice were not included in the calculations. The remaining 4/5 mice per group eventually succumbed to the

infection. In contrast, mice infected with the non-lethal strain of P. yoelii showed no significant effect of treatment with gamma-IFN (Fig. 1B). All the animals recovered from this infection.

A similar experiment was then done in susceptible (Balb/c ByJ and resistant mice (CBA/J). Four of five Balb/cByJ mice receiving diluent died by day 8 after infection with PyL (Appendix item 2, Fig. 2A). Animals treated with either 10,000 or 50,000 U of gamma-IFN/day had diminished parasitemia from days 5-10 ($P<0.005$) and survived a mean of 12 days. Animals infected with the non-lethal variant were also partially protected from infection by gamma-IFN, as shown by lower parasitemia on days 9-14 ($P<0.05$, Fig. 2B). CBA/J mice infected with P. yoelii 17xL showed a slight protective effect with 10,000 and 50,000 U of gamma-interferon/day from days 6-10 (Appendix item 2 $P<0.05$, Fig. 3A). However, there was no effect on mice infected with the non-lethal parasite (21, Fig. 4B).

Further studies were carried out in C57Bl/6J DBA/2 and AKR/J mcie. In each case, gamma-IFN had a protective effect on Pyl but not on PyNL.

3. The role of Pc96, an antigen of P. chabaudi in the erythrocyte membrane of infected mice in cell mediated immunity (23, 24) and its relevance to human malaria (25).

Erythrocyte membranes of P. chabaudi infected mice were purified by affinity chromatography. Mice immunized with these membranes produced antibodies that recognized the erythrocyte membranes of P. chabaudi-infected mice but not normal erythrocytes (23, Fig. 1). Erythrocytes infected with all maturational stages of the parasite were stained.

In order to further characterize this antigen(s), monoclonal antibodies were produced from spleen cells of the immunized mice. Four hybridoma lines were produced which all produced the same immunofluorescence pattern on infected erythrocytes (24, Fig. 1).

To identify the antigen recognized by the immune serum monoclonal the parasitized erythrocytes were subjected to SDS-PAGE, transferred onto nitrocellulose sheets and probed with the immune sera. Only one parasite antigen, the 96 kDa band (Pc96) was detected by immune serum from mice immunized with the erythrocyte membrane preparation) (24, Fig. 2, lane A, compared with normal erythrocytes in lane B). In contrast, polyspecific hyperimmune serum detected numerous parasite antigens in the parasitized erythrocyte extract in lane C, compared with the normal erythrocyte extract in lane D. In addition, two MoAbs, 8F2F9 (IgG1,K), and 6D11E6 (IgM,K) recognize Pc96 (24, Fig. 2, lanes E,F).

Metabolic labelling experiments also indicated that Pc96 was parasite encoded (24, Fig. 3).

In order to determine whether Pc96 was a protective antigen in mice it was purified by affinity chromatography using a monoclonal antibody (6D11E6) as the ligand.

Mice were vaccinated with the affinity purified 6D11E6:Ag and challenged six days after the third booster with blood-induced P. chabaudi infection. Fig. 5 (24) shows parasitemia of the vaccinated and control mice. In all groups, patent parasitemia commenced approximately three days after the challenge inoculation and then rose in parallel for a few days. However, in all the vaccinated mice, parasitemia remained less than 10% and then dropped sharply. In contrast, parasitemia in the control groups continued to rise to 40-50% before declining two days later than the vaccinated groups.

In contrast, passive transfer of purified monoclonal antibodies against Pc96 to mice did not protect them. This suggest that cell-mediated immunity might be the mechanism of protection. Studies are in progress to answer this question.

Sera taken from all the 6D11E6:Ag-vaccinated mice three days before the challenge had very high titers (10^4-10^5) of specific antibody for the membranes of P. chabaudi-parasitized erythrocytes. This antibody activity is species specific since the sera did not cross-react with fixed parasitized erythrocytes from mice infected with P. berghei or P. yoelii (data not shown). Furthermore, in Western blot analysis, all the sera recognized only one antigen of P. chabaudi-parasitized erythrocytes (23, Fig. 2, lanes H-L) with the banding pattern corresponding to Pc96 (lane G).

These results suggested that if an analogue of Pc96 occurred in human malaria, it might be a potential vaccine candidate.

To detect cross-reactivity between Pc96 and antigens of other malarias we used a panel of Pc96-specific sera and MoAb in IFA against erythrocytes from mice infected with P. yoelii or P. berghei, monkeys infected with P. cynomolgi and human erythrocytes infected with P. falciparum or P. vivax. No cross-reactivity was detected with the rodent malarias P. yoelii and P. berghei. However, as shown in 25, Fig. 2, MoAb 7C6 reacted specifically with the erythrocyte membranes of glutaraldehyde- or methanol-fixed erythrocytes infected with P. falciparum (Fig. 2A), P. vivax (2C) and P. cynomolgi (2D). The MoAb produced a perimeter fluorescence on these infected erythrocytes similar to that observed with P. chabaudi-infected erythrocytes. No reaction was seen when P. falciparum (2B), P. vivax (not shown) or P. cynomolgi (2E)-infected erythrocytes were incubated with normal mouse serum.

Western blotting was used to determine the approximate molecular weight of the antigens which cross-react with Pc96. As shown in (25, Fig. 1, MoAb 7C6 detected a 155 kDa band (lane B) in the P. falciparum-parasitized erythrocyte extract, a 222

kDa band (lane C) in the P. vivax-parasitized erythrocyte extract, and a 200 kDa band (lane D) in the P. cynomolgi-parasitized erythrocyte extract. When uninfected erythrocyte extracts were probed with 7C6, no band was detected. Thus, Pc96 shares a cross-reactive epitope with these three primate malaria antigens.

4. Effect of B-thalassemia on malaria-infected mice and the role of the spleen.

Another form of resistance to malaria is that afforded by several hemoglobinopathies. To investigate the protective effects of B-thalassemia against malaria, rodent malaria parasites were studied in C57BL/6J mice with B-thalassemia, in mice in which the thalassemia had been transgenically corrected with the human B^-globin gene, and in hematologically normal mice.

When infection of P. chabaudi adami was studied, parasites were observed in normal and transgenic mice as early as day 7, whereas in thalassemic mice, parasites were not seen until day 11 after inoculation. Similarly, parasitemia peaked on day 13 for normal and transgenic mice, but thalassemic mice did not show peak parasitemia until day 15. In two subseqeunt experiments, this delay in the peak parasitemia was variable (data not shown). Most striking and consistent in all experiments, however, was the observation that the parasitemia in thalassemic mice was only 40% of that attained in the other groups of mice (26, Fig. 1). The differences between the normal mice and the transgenic mice were not significant.

The course of P. chabaudi adami infection in splenectomized normal mice was characterized by a higher-level parasitemia (Appendix item #6, Fig. 3) that occurred on days 13 to 17 after infection. In addition, there was a striking second peak on day 23, with a slow decline through day 43. Normal intact mice were free of the parasites by day 21 without any recrudescence. However, in splenectomized thalassemic mice, parasite growth was paradoxically retarded, and peak parasitemia was not seen until day 19, approximately 5 days later than in intact thalassemic mice. A slow decline in parasitemia occurred thereafter, as in normal splenectomized mice (26, Fig. 3). Baseline reticulocyte counts in uninfected splenectomized mice were not higher than in their intact counterparts (Appendix item #6, Table I) although thalassemic mice displayed reticulocyte counts five to six times higher than those of normal animals. Similarly, during the course of infection with P. chabaudi adami, the increase in polychromatophilic cells was greater in splenectomized mice than in intact mice. (26, Table II).

The behavior of a lethal infection of P. berghei in these mice followed a markedly different course (26, Fig. 4). Within 4 to 5 days after inoculation, parasites were seen in all groups of mice, but unlike the parasitemia seen in the P. chabaudi infections, the

parasitemia in P. berghei infections increased dramatically in the thalassemic mice. In the third and fourth weeks after inoculation, parasites were consistently three to four times more numerous in the thalassemic animals than in normal controls. In addition, differences were seen in the transgenic mice as compared with the controls. Although in general the transgenic mice reacted more like the normal mice than did the thalassemic mice, there were small increases in parasitemia as compared with increases in the normal mice noted in the third and fourth weeks after inoculation that were statistically signigicant. By day 24 after inoculation, the parasitemia exceeded 50%, and by day 30, all animals had died. Survival times in each group were not significantly different.

Discussion and Conclusions

The goal of this study was to determine whether macrophages play an important role in controlling the intraerythrocytic phase of malarial infection. This was accomplished by studying the effect of lethal and non-lethal infections in malaria susceptible and resistant mice. In these models we studied macrophage cytotoxicity, H_2O_2 release and gamma-IFN production. We also studied the effect of recombinant gamma-IFN on the course of these infections. In parallel studies we developed a model of vaccination using an erythrocyte membrane parasite antigen. Other studies looked at the role of the spleen in the genetic resistance to malaria conferred by the B-thalassemic mutation.

Our cytotoxicity studies revealed that at least for ADCC (antibody-dependent cytotoxicity) mice infected with non-lethal malaria had greater activity. This is consistent with our results (discussed below) that macrophages from mice infected with Py17xNL secrete more H_2O_2 than do those from mice infected with the Py17xL. Since ADCC is mediated by H_2O_2 (32) this may explain the increase in activity in the non-lethal infection.

Although we found enhanced cytotoxicity above normal controls in Py17xNL and in P. berghei infection at early time points, macrophages from these mice did not lyse parasitized erythrocytes. However, when P. berghei-infected erythrocytes were presensitized with rabbit anti-mouse erythrocyte IgG, they did release ^{51}Cr, although less than that released by moEIgG (Table I). This result could reflect lysis of only uninfected erythrocytes; however, since the amount of ^{51}Cr released was reduced by less than 50% compared with that from normal mouse erythrocytes, this explanation is unlikely. Another possible explanation is that malarial antigens on the erythrocyte surface are continuously shed. In this regard it is interesting that several erythrocyte membrane malarial antigens must be fixed with glutaraldehyde to be detected, for example, Pc96 and Pf155 (33). It is also possible that although ^{51}Cr was not released from parasitized erythrocytes in the presence of hyperimmune serum, the parasites were damaged or destroyed. Furthermore, the in vitro assay of cytotoxicity cannot approximate the interaction between effector and target cells in an architecturally intact spleen. Our results at this point do not allow us to define the killing mechanism that takes place in vivo.

In these studies we do demonstrate both a difference in the sensitivity of lethal and non-lethal Plasmodial yoelii infections to treatment with recombinant gamma-IFN and differences in the host responses to lethal and nonlethal Py in terms of their own gamma-IFN production and the H_2O_2 response of their splenic macrophages. We explored the gamma-IFN response during PyL and PyNL. The results showed that Balb/cByJ mice infected with PyNL made an immediate gamma-IFN response by their splenic lymphocytes whereas mice infected

with PyL did not. In addition, although BALB/cByJ mice infected with PyNL responded initially, gamma-IFN levels then dropped to control levels where they remained for the duration of infection. In contrast, in the more resistant CBA/J mice there was an initial burst of gamma-IFN production, a fall as parasitemia increased but a recovery to greater than control levels as parasitemia was cleared. This was true for PyNL as well as PyL.

The production of H_2O_2 was monitored in spleen cell cultures at the same time as gamma-IFN production and similar kinetics of production were observed. It is not clear why, even in non-lethal infections, levels of gamma-IFN and H_2O_2 decrease as parasitemia climbs. It will be of interest to determine whether this is due to a shift in cell subpopulations present in the spleen. Such studies are in progress. Nevertheless, these results are consistent with those of Wasencraft, et al (34) who studied nitroblue tetrazolium reduction in macrophages during the initial phase of P. yoelii nigeriensis and found increased activity only to day three and Dockerell, et al (35) who found similar results to those presented here. In the kinetics of macrophage activation using the nitroblue tetrazolium assay.

Our results are also consistent with the findings of others who compared the effects of lethal and non-lethal rodent infections. Brinkmann, et al. (36) found that macrophages obtained from mice infected with P. yoelii (mild) had greater capacity to generate O_2 metabolites than macrophages obtained from P. berghei-infected animals (a lethal infection in mice). Similarly, Taverne et al (37) found that macrophages from mice infected with non-lethal P. chabaudi and P. yoelii (lethal and P. berhgei-infected mice. Our results provide a possible explanation for these findings. We suggest that non-lethal malaria induces a greater gamma-IFN response resulting in macrophage activation for phagocytosis and secretion of toxic oxygen metabolites. These results are consistent with the known effects of gamma-IFN in activating macrophages to release H_2O_2 (30).

The data presented here, suggest, for the first time, that there may be differences in the cellular responses to PyL and PyNL. We observed that mice infected with PyL respond to treatment with recombinant gamma-IFN whereas mice infected with the milder parasite were not appreciably affected by such treatment. Additional experiments indicated that if animals were only pretreated or pretreated and then treatment was continued for 5 days with gamma-IFN, they were not protected, suggesting that the lymphokine needs to be present continuously for a protective effect (not shown). These results suggested to us that injection of gamma-IFN might be compensating for a poor endogenous gamma-IFN response.

Our results also suggest that preferential stimulation of gamma-IFN-secreting lymphocytes in prophylaxis would be a potentially effective immunization regimen. Analysis of how PyNL induces a better gamma-IFN response may help identify the appropriate antigens

or vaccination regimens to elicit such a response. Finally, gamma-IFN itself may help induce a protective immune response since it has recently been shown to be a potent adjuvant in immunization against PyL (38).

Another vaccine model was also developed in P. chabaudi. In these studies we demonstrate that Pc96 is a parasite-encoded antigen of M_r 96000 on or in the surface of P. chabaudi-infected erythrocytes. Partial protective immunity against P. chabaudi could be induced in mice using 6D11E6:Ag, the parasite antigen affinity-purified with Pc96-specific MoAb. Although 6D11E6:Ag contained other proteins in addition to the 96 kDa, the data strongly suggest that Pc96 is the only parasite immunogen in the preparation since mice vaccinated with 6D11E6:Ag produced antibodies only against Pc96 and its fragments.

The protective immune mechanisms induced by Pc96 remain to be determined. Passive immunization with MoAb did not protect mice against P. chabaudi (data not shown); however, since MoAbs are epitope specific this does not exclude the possible role of antibodies specific for other epitopes in protection. Studies of passive immunization with sera and T-lymphocyte subsets, should help to elucidate the immune effector mechanisms involved in protective immune response to Pc96.

Our studies also demonstrated that antigens which cross-react with Pc96 are present in the primate malarias, P. falciparum, P. vivax, and P. cynomolgi. The corresponding molecules are Mr 155,000, 222,000 (Pv222), and 200,000 (Pcy200) respectively.

Since these antigens share the same topographic location as Pc96 and a similar requirement for fixation for the IFA reaction, it is possible that they are homologous antigens. Thus, the same immune responses may be elicited as for Pc96, which induces protective immunity against P. chabaudi infection.

The fact that four antigens of different species of malaria share the same topographic location and at least one serological cross-reactive epitope is of interest. It is possible that this antigen serves a function essential to the biology of the parasite and the cross-reactive epitope plays a key role in the function of the molecule.

Our studies in B-thalassemic mice open a new area of research, namely, the use of genetic mutations of erythrocytes in vivo to study the requirements for parasite invasion and growth.

The role of the spleen and function of macrophage in this mutation is complex. Clearly, differences in rates of parasitemia are not related to changes in the reticulocyte count in splenectomized animals (26, Tables I and II). As expected, parasitemia was of a higher level and was more prolonged in

the normal splenectomized animals. This is consistent with the well known role of the spleen as a filter for red cell inclusions. The prolongation of the parasitemic period was also noted in splenectomized thalassemic mice, but the delay in achieving the maximum parasitemia in splenectomized animals was unexpected and points to a paradoxical permissive role of the B-thalassemic mouse spleen. This possible permissive effect intact thalassemic mice is also independent of any reticulocyte effect. Further experimentation will be required to understand this phenomenon.

More recent studies have utilized mice with spherocytic, hemolytic anemia which were completely resistant to P. chabaudi infection. The mechanism of this resistance is not known as yet.

To summarize briefly, our studies point to an important role for activated macrophages in control of malarial infections and in possible vaccination strategies. Why non-lethal infections elicit a better macrophage response is the subject of future studies (39).

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Appendix

Legends to Figures

Fig. 1. Lymphoproliferative responses to PyL antigen in Balb/c ByJ (upper panel) and CBA/J (lower panel) mice during PyNL and PyL. Data represent the mean ± SE.

Appendices: Enclosed.

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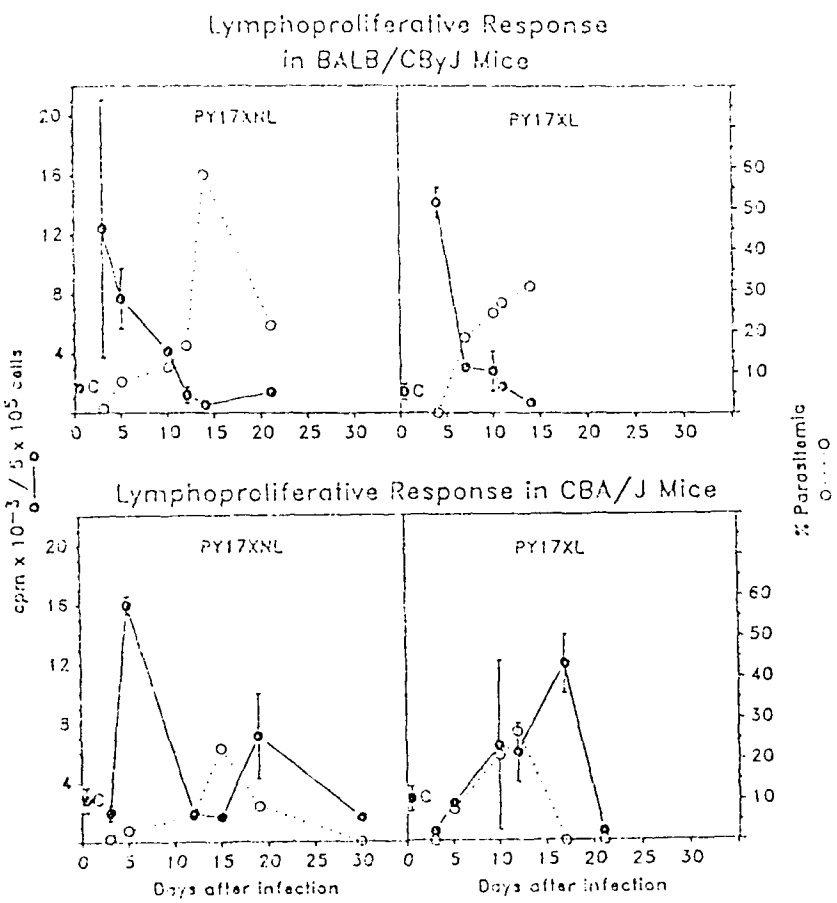
Fig 1


Lymphoproliferative Response
in BALB/CByJ Mice
PY17XNL
PY17XL
Lymphoproliferative Response in CBA/J Mice
PY17XNL
PY17XL
cpm x 10⁻³ / 5 x 10⁵ cells
% Parasitemia
Days after infection
Days after infection